CLS 291 Clinical Hematology 1

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Lecture 1

# Blood Collection and Anticoagulants

# Outline

1. The process of phlebotomy.
2. Equipment of venipuncture.
3. Difference between blood samples obtained from venous and capillary.
4. Causes of misleading results related to specimen collection.
5. Commonly used containers in the hematology lab.

# The Equipment for Venipuncture

- It is now common practice for specimen collection to be undertaken by specially trained **phlebotomists**.

- **Items to be included in a phlebotomy tray :**

  - Syringes and needles:
    - 19G and 21G needles are suitable for adults.
      - 19G = 1.1 mm; 21G = 0.8 mm
    - 23G is used for children.
      - 23G = 0.6 mm.
  - Tourniquet
  - Specimen containers
  - Sample request form
  - Alcohol swabs
  - Sterile gauze swabs
  - Self-sealing plastic bags (biohazard sample bag)
  - Rack to hold specimens upright during the process of filling.

Phlebotomy tray

# The Equipment of Venipuncture

Gloves

Needles

The Hub

Evacuated Collection Tubes


Alcohol Wipes

Syringes

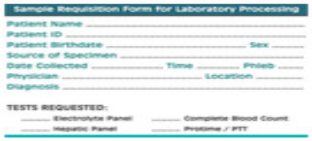
Bandages/Tape

Gauze Sponges

Tourniquet

Sharps Container


Povidone/Iodine Swabs/Wipes



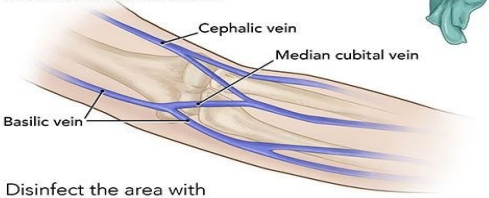

Requisition Form

# Phlebotomy Procedure

## Venipuncture
Blood collection procedure guide

Gather all equipment, wash hands, and put on sterile gloves.

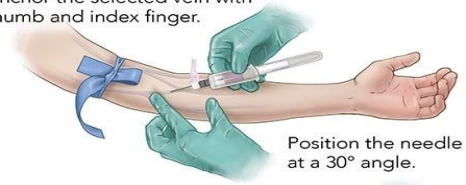
Ask the patient to make a fist and select the venipuncture site in the antecubital fossa.




Disinfect the area with a 70% alcohol swab, working from the center outwards.

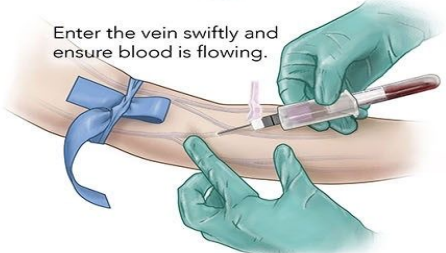
Apply a tourniquet about 3 to 4 inches above the site.

Anchor the selected vein with thumb and index finger.

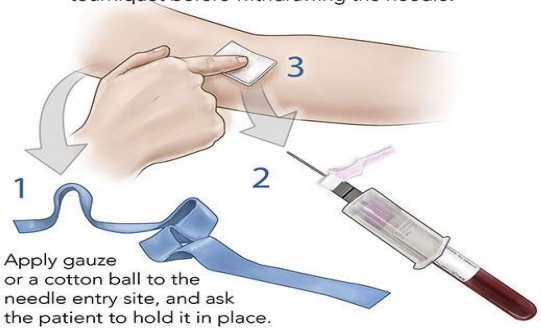


Position the needle at a 30° angle.

Enter the vein swiftly and ensure blood is flowing.



After blood has been collected, release the tourniquet *before* withdrawing the needle.




Apply gauze or a cotton ball to the needle entry site, and ask the patient to hold it in place.

Remove and immediately invert the tube 8 to 10 times to mix the sample with the tube additives.

Discard the used needle in the sharps container.

Remove gloves and wash hands with soap and water.

Label the tube for transport to the lab, indicating:
- Patient's full name
- Patient ID
- Birth date
- Date of sample.

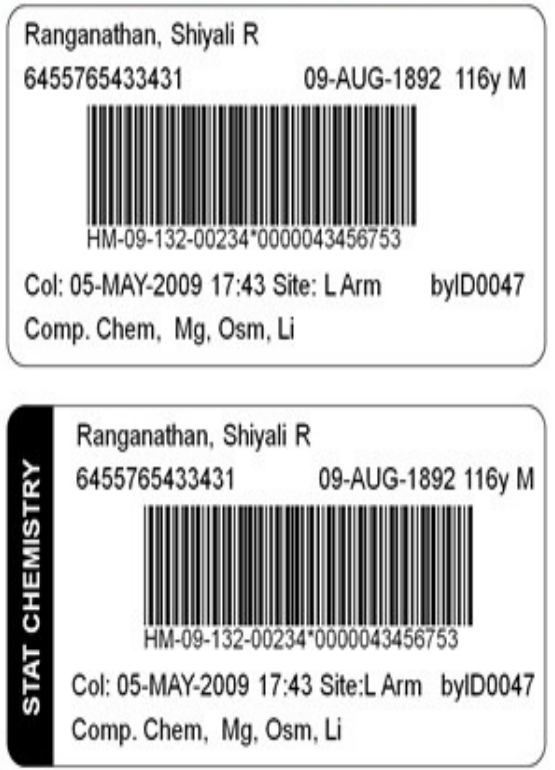
For more information, visit:
World Health Organization Guidelines on Drawing Blood: Best Practices in Phlebotomy

# Capillary Blood

- Skin puncture can be used to obtain a small amount of blood.
- These methods are mostly used when it is not possible to obtain venous blood (e.g., in infants under 1 year, in gross obesity)
- For Point of Care (POC) blood tests.
- DIFFERENCES BETWEEN CAPILLARY AND VENOUS BLOOD
  - Capillary Blood:
    - Contains some **interstitial and intracellular fluid.**
    - **Shows slightly higher** packed cell volume/ haematocrit (PCV/Hct), red blood cell count (RBC) and haemoglobin concentration (Hb).
    - **Higher** total WBCs count.
    - **Lower** platelet count.

# Information on the Blood Tube

- Barcode
- Patient's medical record number (MRN)
- Sample number
- Collection date



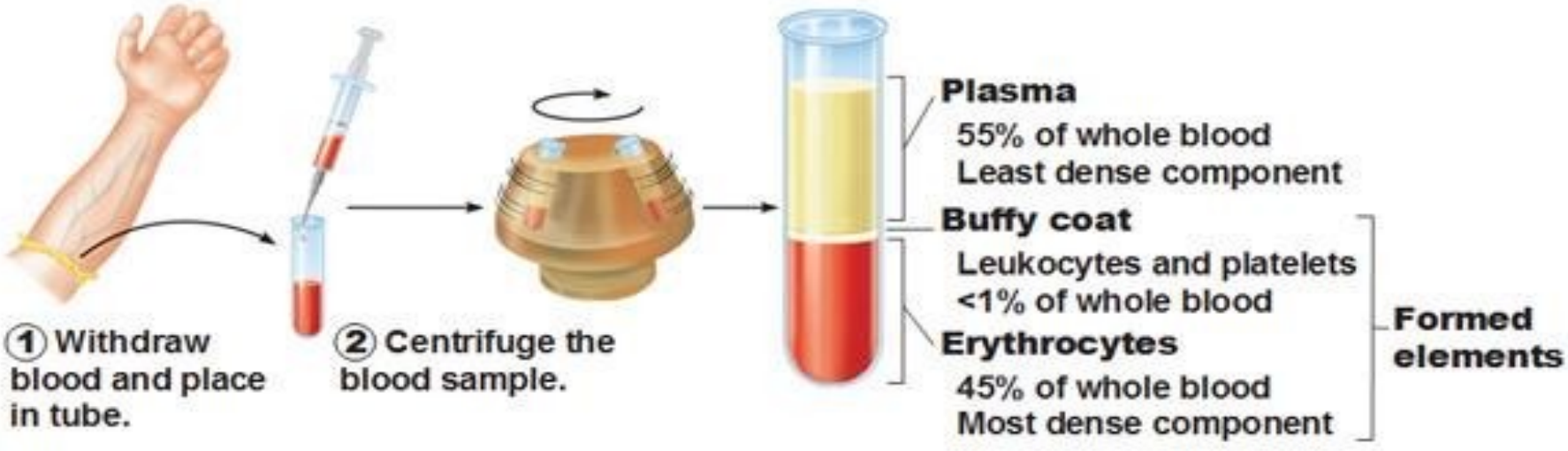
# Blood Collection (Phlebotomy)

- Cautions should be applied before, during, and after specimen collection.

| Causes of misleading results related to specimen collection |
|---|
| **Pre-collection** |
| • Smoking Stress<br>• Drugs or dietary supplement administration within 8 h. |
| **During collection** |
| • Incorrect type of tube<br>• Posture: lying, standing or sitting |
| **Handling of specimen** |
| • Excess anticoagulant<br>• Inadequate mixing of blood with anticoagulant<br>• Error in patient and/or specimen identification<br>• Inadequate specimen storage conditions<br>• Delay in transit to laboratory |

# Blood Component

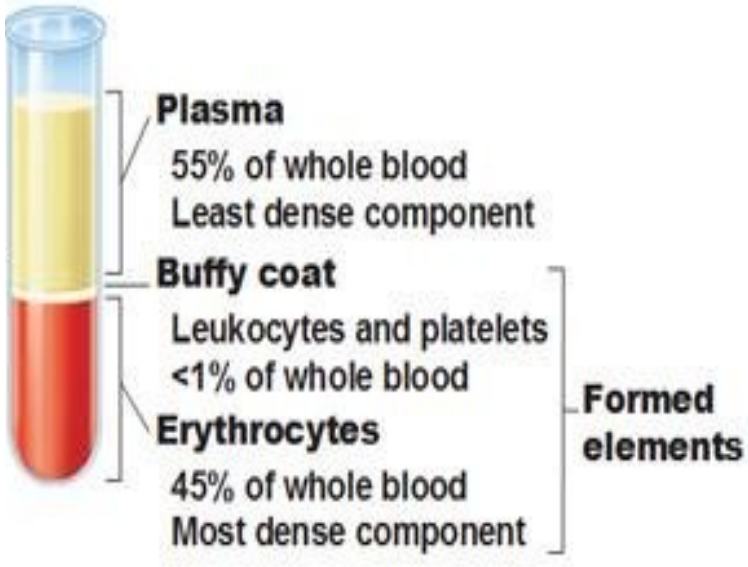
## Major Components of Whole Blood

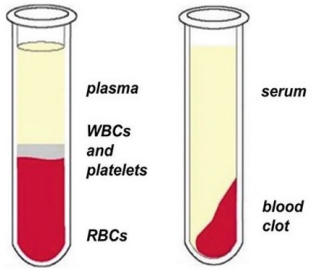


# Blood Component

Blood can be divided into two parts:

- Cellular part: White blood cells, Red blood cells, and platelets.
- Non-cellular part (Plasma): water, nutrients, salts and waste products, and coagulation factors.
- **Plasma** is the liquid portion of blood, it constitutes about 55 % of blood volume and 90% of plasma is water.
- **Serum** resembles plasma in composition but lacks coagulation factors.



# Specimen Containers

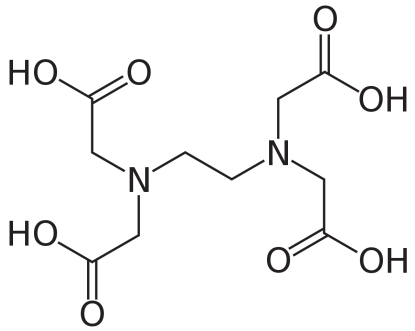
Blood tubes used mainly for hematological testing are:

1. Ethylenediamine tetracetic acid (EDTA) tube (purple cap)
2. Sodium citrate tube (light blue cap)
3. Heparin (green cap)

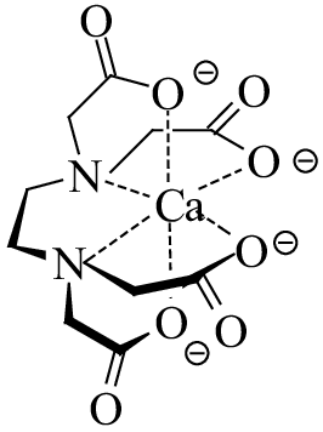
# Specimen containers

## Ethylenediamine tetraacetic acid (EDTA) tube (purple cap)

- Uses:
  - EDTA anticoagulant is used for blood counts.
- Mechanism:
  - EDTA removes calcium, which is essential for coagulation.
  - Calcium is either precipitated as insoluble oxalate (crystals of which may be seen in oxalated blood) or bound in a non-ionized form.
- Effect of excess EDTA:
  - Decrease in PCV (↓ PCV)
  - Red cells, and leukocytes, are both affected.
  - causing shrinkage and degenerative changes.
  - The platelets may also be affected; an excess of EDTA causes them to swell and then disintegrate, causing an artificially high platelet count, as the fragments are large enough to be counted as platelets.



EDTA structure



Calcium EDTA chelate structure

# Specimen Containers

## Sodium citrate tube (light blue cap)

- Uses:
  - Mainly used for coagulation testing (the ratio is 1:9).
  - Erythrocyte sedimentation rate (ESR) test (ratio 1:4).

- Mechanism:
  - Sodium citrate works on the same mechanism as EDTA anticoagulant in which they both contain citrate that chelates (removes) calcium, which is essential for coagulation.

### Why don't we use EDTA?

- Sodium citrate is best used for coagulation tests since factors V and VIII are more stable in this substance than EDTA.
- EDTA binds irreversibly to calcium, and its chelation properties continue with the calcium ions binding even if more calcium is added, like in coagulation tests. Thus, it is not a suitable anticoagulant for coagulation testing. In contrast, Sodium citrate binds reversibly to calcium without causing interference in coagulation testing.

# Specimen Containers

## Heparin (green cap)

- It does not alter the size of the red cells.
- Reduce cell lysis after blood withdrawal.

- Uses:
  - Osmotic fragility tests. <<< minimum lysis with heparin
  - Immunophenotyping.

- Avoid using heparin for:
  - Blood counts and blood films.
    - Because it induces platelet and leucocyte clumping.
    - And gives a faint blue coloration to the background.

- Mechanism:
  - Heparin binds to antithrombin, thus inhibiting the interaction of several clotting factors.